

Yeast Showdown 1: Image processing

We are interested in how signaling pathways evolve and how cells integrate multiple signals. To study this we use budding yeast. In the experiment we will analyze here, we compare the response of two different strains of yeast both grown in a mixture of 0.5% galactose and 0.0625% glucose. Both strains have a yellow fluorescent reporter (YFP) that is expressed when cells respond to galactose – the promoter driving YFP is that of a gene needed for galactose metabolism. To minimize variability we co-culture the two different strains thereby ensuring that both strains of yeast are exposed to the same external environment. In order to differentiate between the two strains, one strain constitutively expresses a red fluorescent protein (RFP) while the other strain constitutively expresses a blue fluorescent protein (BFP).

Experiment:

You inoculate a tube of growth media with both yeast strains, let them grow, and image the cells under the microscope. You take separate images for differential interference contrast (DIC), RFP, BFP and YFP. The DIC image does not excite the fluorophores – all cells look similar. The RFP, BFP, and YFP pictures use special filters to excite and detect the respective fluorophore. Now, you want to determine if the two strains differ in their response to galactose.

There are four images corresponding to time 2 in the time course experiment:

DIC_Time2, RFP_Time2, BFP_Time2, and YFP_Time2.

1. To explore the data:
 - a. Visualize each of the images separately.
 - b. If you overlay the fluorescent images, what do you expect to see? Overlay them to form a single image. Because of camera settings, each of the different images can be on different intensity scales; make sure to normalize each image so that each image contains pixel from a minimum of 0 to a maximum of 1. Are there any colors that you didn't expect to see? How could this happen?
2. Do the pixels that come from RFP or BFP cells have a higher YFP value? What's the mean and standard deviation of each group of pixels?

Extras:

1. Is the difference in YFP intensity significant?
2. Find the background YFP level for the image (the YFP values from outside the cells). When you take into account the background is the difference still significant?

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